

### **REMARKS**

Applicants thank the Examiner for the thorough consideration given the present application. Claims 1-8 are currently being prosecuted. The Examiner is respectfully requested to reconsider her rejection in view of the remarks as set forth below.

#### **Rejection under 35 U.S.C. 103**

Claims 1-8 stand rejected under 35 U.S.C. 103 as being obvious over Stanley et al. (US Patent 3,909,358) in view of Odell (US Patent 4,532,212) and the admitted prior art of the present specification. This rejection is respectfully traversed.

Applicants wish to first point out that the present invention is a process for preparing lysozyme by a highly selective adsorption of lysozyme using adsorbent kaolin diatomaceous earth and zeolite and then eluting the adsorbed lysozyme from the adsorbents using salt solution. Not only can the lysozyme be simply and effectively recovered at a 95% rate, but the egg white is not contaminated with any chemicals or solvents so that it may be used in food processing after the removal of the lysozyme. It should be remembered that the lysozyme is removed from the egg white by the adsorbent, which does not affect the egg white. The elution using salt solution is then only performed on the adsorbent and adsorbed lysoszyme leaving the egg white pure. In addition, the present process also does not cause any problems with environmental pollution.

The Examiner states that the Stanley et al. reference teaches a process of preparing an enzyme including mixing a starting material with diatomaceous earth and also a treatment with salt solution is disclosed. Stanley et al. also discloses that lysozyme is an enzyme which can be prepared. The Examiner relies on Odell to teach that the egg white is a source for lysozyme and that purification techniques such as crystallization and resin techniques such as chromatographic separation are also possible. The Examiner relies on the admitted prior art to teach a method of purifying lysozyme by using an adsorbent such as bentonite. The Examiner feels that it would have been obvious to one of ordinary skill in the art to use the egg whites of Odell in the Stanley

et al. process of treating with diatomaceous earth and treatment of the sodium chloride solution and to purify the enzyme using crystallization or resin treatment as disclosed in Odell so that the lysozyme is adsorbed using diatomaceous earth as disclosed in the admitted prior art.

Applicants disagree with the Examiner's understanding of the various references and disagree that the present invention is obvious over these references.

First, in regard to Stanley et al., the process disclosed there is an enzyme insolubilization (also referred to as enzyme immobilization). Thus, the aim of this reference is not to separate lysozyme but instead to immobilize an enzyme. The enzymes are connected to a chitin carrier through an enzyme-chitin-glutaraldehyde reaction and the resultant reaction product is a well known Schiff-base formation technique in the field of immobilized enzyme technology. Further, the Examiner has indicated that this reference teaches mixing a starting material with diatomaceous earth. However, Stanley et al. makes it clear that the purpose of the diatomaceous earth is to increase porosity so as to provide a liquid-permeable mass. This is completely different than the use of diatomaceous earth in the present invention, where it is used to adsorb enzymes. Thus, Applicants submit that Stanley does not disclose any process of preparing lysozyme and the use of diatomaceous earth is for completely different reason than that of the present invention. Further, Applicants submits that it would not be obvious to use the diatomaceous earth in a manner, such as in the present invention, based on the disclosure of Stanley et al.

Furthermore, the salt solution used in Stanley et al. is employed for washing out the impurities and is not used for eluting an enzyme. Thus, this teaching of a salt solution is likewise not relevant to the present application. Further, even though Stanley et al. mentions that lysozyme is an enzyme, its only concern is for enzyme immobilization, which is completely different than the enzyme purification of the present application.

Concerning the Odell reference, Applicants agree that it is common knowledge that egg white is a source of lysozyme. However, Odell only suggests that the protein or enzyme can be

dissolved in organic solvents or in aqueous organic solvent mixtures which make it possible that fraction precipitation and/or crystallization, chromatographic or electrophoretic techniques can be applied. Odell further teaches the experimental data that lysozyme is soluble in methanol. It is clear that the method of Odell is not appropriate for the present invention since the use of an organic solvent would ruin the resultant egg whites and further would cause environmental pollution. It should be remembered, that it is important to keep the egg white pure so that it could still be used as a food ingredient after the lysozyme is isolated.

Accordingly, Applicants submit that Odell does not teach the concept of preparing lysozyme by mixing an egg white with an adsorbent to adsorb the lysozyme and then to elute the adsorbed lysozyme with a salt solution.

The Examiner also refers to the admitted prior art to teach the idea that bentonite can be used as an adsorbent for lysozyme. The Examiner indicates that the lysozyme was not eluted. In fact, the first paragraph on page 3 of the specification makes it clear that while bentonite can adsorb lysozyme, it is not easily eluted and requires a solution of 5% of acetic acid in pyridine. Because of the difficulty of eluting the lysozyme from bentonite, Applicants submit that this admitted prior art actually teaches against the possibility of selective adsorption. Further, as is indicated in table 1 in the present application, bentonite generally adsorbs a number of proteins rather than selectively adsorbing lysozyme from egg white. In fact, an effective method of recovering lysozyme which is adsorbed by bentonite is yet to be discovered.

The Examiner has further stated that the claims differ from Stanley et al. in that the use of naturally occurring oxides are not disclosed as being useful for adsorbing lysozyme. Applicants also wish to point out that not all naturally occurring oxides can selectively adsorb lysozyme from egg whites. Applicant has conducted countless experiments and with enormous efforts to discover that the minor component protein of lysozyme (about 3.5% of the total dry weight of the egg) can be adsorbed specifically from the whole egg white by using kaolin, zeolite and diatomaceous earth.

The examiner has stated that it would be obvious to one skilled in the art to use egg whites as a lysozyme source as taught by Odell in the Stanley et al. technique of treating with diatomaceous earth mixtures and treatment with sodium chloride solution to purify the enzyme using crystallization or resin treatment as disclosed by Odell and adsorbing the lysozyme using the diatomaceous earth disclosed by the admitted prior art. Applicants submit that the present claimed invention is not obvious for the reasons recited above. Applicant submit that the combination of these three references still does not teach a workable solution for adsorbing lysozyme from egg whites then eluting the adsorbed lysozyme with a salt solution. As indicated above, Stanley et al. uses diatomaceous earth for a different purpose. The salt solution is not used to elute in Stanley et al. The showing of the admitted prior art is not involved in material which permits elution. Accordingly, Applicants submit that combination of these teachings would not render the present invention obvious.

Further, there is no motivation shown for combining these various teachings and in fact the teachings seem to teach against their combination. Thus, the Examiner suggests that the technique of the admitted prior art can be used with the diatomaceous earth of Stanley et al. However, the diatomaceous earth in Stanley is not used for the same purpose. Further, the admitted prior art teaches against this type of arrangement since bentonite does not allow for easy elution. Accordingly, Applicants submit that no motivation is present.

For these reasons, Applicants submit that claims 1-8 are not obvious over the combination of references suggested by the Examiner.

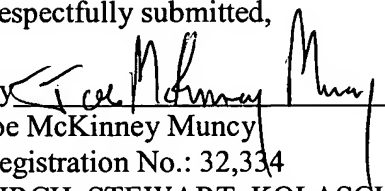
Conclusion

In view of the above, it is believed that the claims clearly distinguish over the patents relied on by the Examiner either alone or in combination. In view of this, reconsideration of the rejection and allowance of all of the claims is respectfully requested.

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Respectfully submitted,

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